# Analysis of Differences in Functional Groups in Green Tea Kombucha (Camellia sinensis) and Rosella Flower Kombucha (Hibiscus sabdariffa) Using FTIR Spectrophotometer Instrument

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Keywords: Kombucha, Green Tea, Rosella Flower, FTIR, Functional Groups.

Abstract: Functional groups are groups that determine the properties of organic compounds. Kombucha is a fermented drink made from tea and sugar to which a symbiotic culture of bacteria and yeast (SCOBY) is added. The aim of this study was to analyze the differences in functional groups found in green tea kombucha and rosella flower kombucha (*Hibiscus sabdariffa*). This research includes experimental research by analyzing functional groups in test samples using the FTIR (*Fourier Transform Infrared Spectroscopy*) instrument. The results of the study showed that green tea kombucha has functional groups C-N amine, C=C alkene, C=C (triple) alkyne, and O-H alcohol, while the functional groups of rosella kombucha tea are produced as many as 5 functional groups, including the functional group C - O (Alcohol, ether, carboxylic acid, ester).

## 1 INTRODUCTION

Tea is a beverage product that has many benefits for the human body. Tea plants grow in tropical and subtropical regions such as Indonesia. The chemical compounds contained in tea are catechins, vitamin E, vitamin C, tannins, flavonoids, theophylline, polyphenols, and a number of minerals such as Mg, Ge, Mo, Se and Zn. Although from the same plant. What distinguishes all these types of tea is the manufacturing process (Arisudin *et al.,* 2021). Tea has many health benefits so it is developed into various derivative products, one of which is kombucha.

Kombucha comes from a mixture of sweet tea fermented by a number of microorganisms, especially yeast and acetic acid bacteria (Toeh *et al,* 2004). This type of tea has advantages when compared to the usual type of tea because kombucha tea contains organic acids and several organic compounds and amino acids. Because these contents are effective as antioxidants and antibacterials to improve digestive tract function and increase body resistance and are also able to lower cholesterol levels, blood pressure and improve liver function and reduce the spread of cancer (Sumanto *et al.,* 2023).

Tea has several types, one of which is green tea, which can be turned into kombucha for research because kombucha is a fermented drink that has various health benefits that are different from ordinary green tea. Fermenting green tea with kombucha produces probiotics, enzymes, and organic acids that are not present in pure green tea. Kombucha research allows scientists to explore these additional benefits, such as improved digestive health, detoxification, and potentially more powerful antimicrobial and antioxidant properties. In other words, converting green tea into kombucha enriches the beverage with bioactive components that can provide broader health effects (Villarrral *et al,* 2018). Several methods are required to make kombucha tea.

The main ingredients needed to make kombucha include tea, sugar, and an initial liquid containing kombucha starter culture (Toeh *et al,* 2004). Green tea kombucha has a variety of widely recognized health benefits, including antioxidant, antimicrobial, and digestive health-promoting activities. Green tea kombucha, a fermented green tea with microbes, offers the added benefit of containing a variety of organic acids, vitamins, and other bioactive compounds. This study used samples of kombucha tea from rosella flowers because rosella flowers contain several secondary metabolic compounds that are beneficial for body health. To utilize the functional properties of rosella and the ability of microbes in fermenting kombucha which produces compounds that are beneficial for the body, rosella flowers are transformed into kombucha tea products which are expected to provide greater benefits for the general public.

The rosella plant (*Hibiscus sabdariffa*) comes from the Malvaceae family or fiber-producing plants. Rosella is one of the plants that is familiar to the public, because almost all parts of this plant can be used, especially for health (Baniu & Olii, 2024). This rosella plant contains calcium, vitamins C, D, B1, B2, magnesium, omega-3, beta-carotene, and 18 essential amino acids, including lysine and agrin. Vitamin C in rosella flowers is three times more than in black grapes, nine times more than in citrus fruits, and ten times more than in fruit (Wijayanti & Retnaningsih, 2023). However, there are several research gaps that require the latest innovations to deepen understanding and maximize the benefits of green tea kombucha. In the research of Arisudin *et al* (2021), further research is also needed to understand how green tea kombucha affects body cells and tissues at the molecular level and studies are needed that compare the health effects of kombucha from various types of tea to determine the specific advantages of green tea. Meanwhile, in the research of Sumanto *et al* (2023), further research is needed to determine the optimal fermentation conditions that produce maximum health benefits. A different study was also conducted by Inggrid *et al* (2019). This study did not explore the interaction between green tea and additional ingredients such as rosella flowers in kombucha fermentation, so further research is needed to understand how this combination affects the chemical profile and health benefits of kombucha.

From several research results that have been conducted, many show that one of the plants that has antibacterial activity is the rosella plant. Rosella (*Hibiscus sabdariffa L.*) is a plant that has antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antihypertensive and antifungal activities. The antimicrobial properties of rosella flowers are indicated by polyphenol compounds such as flavonoids, namely anthocyanins and gosypetin, phenolics, tannins and saponins. Research conducted by Mukani (2014) also stated that giving rosella flower kombucha increased erythrocyte levels and hemoglobin levels in the blood of mice. To determine the compound content in rosella flower kombucha and green tea, functional group analysis was carried out on rosella flowers and green tea using the *Fourier Transformed Infrared* (FTIR) Spectrophotometry instrument method.

*Fourier Transformed Infrared* (FTIR) spectrophotometer is an instrument used to detect, identify, and analyze a type of functional group from the sample being tested without damaging the sample (Abriyani *et al.,* 2024). FTIR spectroscopy can analyze the presence of mixtures in samples without damaging the sample to be analyzed. Infrared spectrophotometer (FTIR) is generally used to identify organic bioactive compounds, especially in plant extracts. Bioactive compounds contained in plant extracts have their own advantages, where almost all of the compounds show absorption power against infrared radiation and FTIR has non-destructive properties, meaning properties that do not easily damage samples so that it is possible to carry out measurements in situ (Nurhamidah *et al.,* 2024).

Through FTIR testing on green tea kombucha and rosella flower kombucha, we can gain a deeper understanding of the chemical composition and molecular structure of the two types of kombucha, to determine the contents therein, such as polyphenols, organic acids, and vitamins. By comparing the composition between the two types of kombucha we can find out about the contribution of additional ingredients, such as rosella flowers, to the chemical profile of kombucha. In addition, through molecular structure analysis, we can explore the interactions of compounds in both types of kombucha during the fermentation process. Thus we can know the overall properties and health benefits of kombucha (Jati & Cahyanto, 2020).

The basic principle of IR spectrophotometric analysis is through the absorption of electromagnetic radiation by certain functional groups, from the absorption process a readable absorption spectrum is formed, from this spectrum it can be used to determine the functional groups contained in a compound (Pristiwani & Ridwanto, 2023). The infrared region of the electromagnetic wave spectrum starts from a wavelength of 14000 cm- to $10^{-1}$. Infrared spectroscopy analysis includes several methods based on the absorption or reflection of electromagnetic radiation. When a compound is placed in an infrared beam, the absorbed energy causes changes in bond vibrations. The resulting infrared spectrum is complex data information, so it can comprehensively describe the chemical characteristics of a sample (Abriyani *et al.,* 2024).

The working mechanism of FTIR is that the light coming from the light source will be transmitted, then split by the beam splitter into 2 perpendicular rays. In a spectrophotometer there are 2 types of mirrors, namely a stationary mirror and a moving mirror which function to reflect light. The resulting light from the reflection of the two mirrors will be reflected back to the beam splitter to react again. The 2 rays that were divided earlier will be partly directed towards the sample and partly towards the source. The back and forth movement of the mirror causes the incoming light to reach the detector and fluctuate. The back and forth movement of the mirror causes the incoming light to reach the detector and fluctuate. The fluctuation of light that reaches this detector will produce a signal on the detector called an interferometer. This interferometer will be converted into an IR spectra with the help of a computer based on mathematical operations (Abriyani *et al.,* 2024).

The FTIR measurement technique is a standard method for characterizing the molecular structure of organic materials. From this characterization, an absorption spectrum is obtained which describes the interaction between electromagnetic electric field radiation (IR radiation) and the electric dipole moment of the molecule. Each absorption peak in the spectrum is related to the excitation of the vibrational mode of the molecule concerned, which consists of stretching and bending vibrations such as out-of-plane bending (twisting, wagging) and in-plane bending (rocking) (Raturandang *et al.,* 2022).

# 2 METHODS

## Study Location

The practicum entitled Analysis of differences in functional groups in green tea kombucha (*Camellia sinensis*) and rosella flower kombucha (*Hibiscus sabdariffa*) using an FTIR spectrophotometer instrument was carried out at the Instrumentation and Measurement Laboratory of Sunan Ampel State Islamic University, Surabaya, District. Gunung Anyar, Surabaya.

## Tools and materials

The tools used in this practicum are a drop pipete and FTIR spectrophotometer which are connected via computer. Meanwhile, the materials used are tissue, green tea, and rosella flower kombucha tea.

## Ways of working

This research uses qualitative analysis methods. The subjects of this research used 2 samples, namely green tea and rosella flower kombucha tea. The tool used in this research is FTIR (*Fourier Transform Infrared*). The qualitative data processing and analysis method was carried out by analyzing the data through the FTIR test to detect the presence or absence of functional groups in the contents of green tea samples and rosella flower kombucha samples.

The way it works is to turn on all the equipment, both the FTIR instrument and the computer, then when the computer is on, click the microlab menu on the computer, enter the password in the menu, then click start, method, new, click data collect only, then click instrument (click full in the spectral range), then click save as. The next step is to create a folder by clicking check and activate, then click start then the next step is to wet the instrument on the sample holder with alcohol and clean it with a tissue, then samples of rosella flower kombucha tea and green tea are taken using a dropper and dropped on the sample holder. Which has been cleaned with alcohol, then click next on the computer and the spectra will appear on the computer screen, after that click data handling, then export, CPS and change the name. The final step is to print the spectra data for samples of rosella flower kombucha tea and green tea in the form of word or PDF, then save the file.

# 3 RESULTS and DISCUSSIONS

The results of the analysis of green tea functional groups using an infrared spectrophotometer (FTIR) provide absorptions in the wave number area (cm-1) which can be seen in Figure 1.

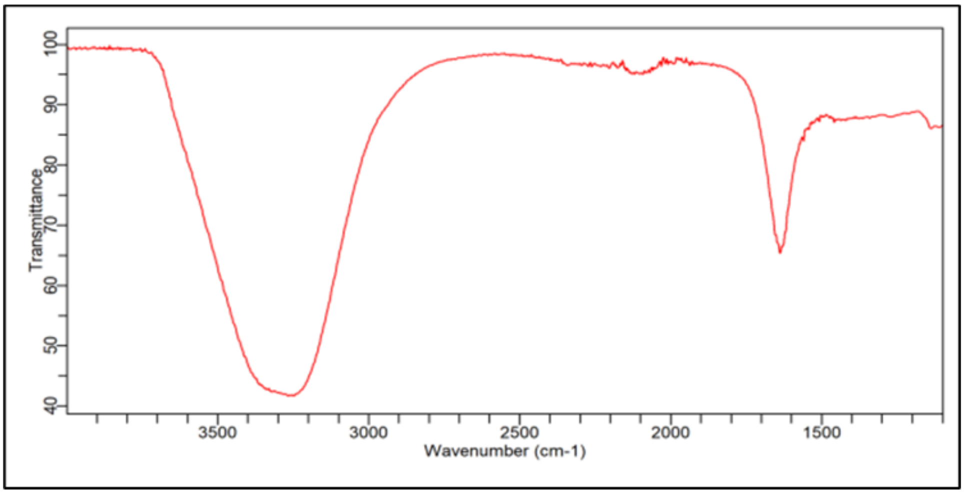


Figure 1: Green Tea Analysis Results

The results of the wave number absorptions using FTIR are then interpreted in Table 1 below

| Wave Number ($cm^{-1}$) | | Intensity | Functional groups |
|---|---|---|---|
| **Theoretical** | **Results FTIR** | | |
| 3000-3700 | 3267,01 | Wide | O-H |
| 1500-1675 | 1638,16 | Sharp | C-C aromatic |
| 1000-1300 | 1136,83 | Currently | C-O |

The absorption results showed the presence of certain functional groups in the green tea samples. Functional group analysis uses FTIR with a wave number limit of 1000-4000 cm-1. The wave number absorption results in this green tea sample are at a wave number of 3267.01 cm-1 with a wide intensity and 1638.16 cm-1 with a sharp intensity. The wave absorption at 3267.01 cm-1 with a wide intensity indicates the presence of the O-H functional group, while the wave absorption at 1638.16 cm-1 with a sharp intensity indicates the presence of the aromatic C-C functional group.

The results of functional group analysis on rosella samples can be seen in Figure 2 and the interpretation in Table 2.

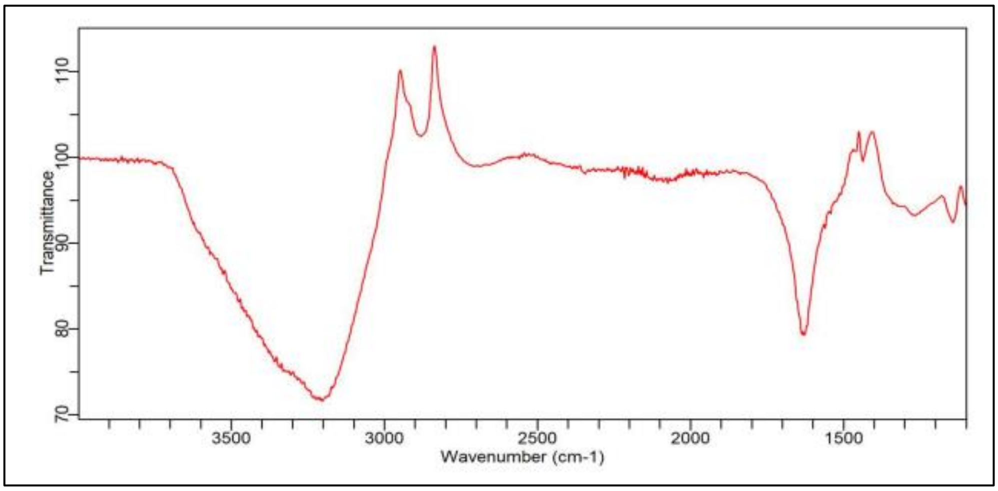


Figure 2: Results of Rosella Kombucha Tea Analysis

Table 2: Interpretation of FTIR Spectra of Rosella Kombucha Tea

| **Wave Number ($cm^{-1}$)** | | **Intensity** | **Functional groups** |
|---|---|---|---|
| **Theoretical** | **Results FTIR** | | |
| 3000-3700 | 3201,78 | Wide | O-H |
| 1500-1675 | 1638,85 | Sharp | C-C aromatic |
| 1000-1300 | 1142,92 | Currently | C-O |
| 1180-1360 | 1265,79 | Currently | C-N (amida, amina) |

The wave number absorption results in the green kombucha rosella tea sample were at wave numbers 3201.78 $cm^{-1}$ with wide intensity, 1638.85 $cm^{-1}$ with sharp intensity, 1142.92 $cm^{-1}$ and 1265.79 $cm^{-1}$ with medium intensity, The wave absorption at 3201.78 $cm^{-1}$ with a wide intensity indicates the presence of the O-H functional group, while the wave absorption at 1638.85 $cm^{-1}$ with a sharp intensity indicates the presence of the aromatic C-C functional group. The results of FTIR analysis on rosella kombucha tea contain almost the same functional groups as green tea.

Green kombucha tea and rosella kombucha tea both contain OH groups. However, the stretch of the transmittance band in rosella kombucha tea was wider than in green tea kombucha. Basically, tea samples contain aromatic OH and C=C groups which are characteristic of flavonoid compounds (catechins) in green tea leaves (Sukaesih, 2021). According to Abriyani *et al.,* (2023) the standard for flavonoids is OH (hydroxyl) wave absorption at 3372 $cm^{-1}$. In general, catechins are found in young tea leaves. The concentration of catechins in tea leaves will decrease as the age of the tea leaves increases. (Nugraheni *et al.,* 2022).

In the Rosella kombucha sample, the stretch in the OH group is wider because the content is influenced by microbiological activity in the manufacturing process. The process of making kombucha uses a 7-21 day fermentation process using the help of microbes, one of which uses the yeast *Saccharomyces cerevisiae* (Priyono & Riswanto, 2021). The alcohol content in kombucha from several sources can be seen in Table 3. The basic content of tea which already contains OH groups (catechins) is added to the alcohol content as an effect of the fermentation process, making the FTIR spectrum stretch wider in the wave number range of 3000-3700 $cm^{-1}$. The spectral stretch at 3201.78 $cm^{-1}$ is also influenced by the flavonoid and phenolic content in rosella leaves. Phenolic compounds are compounds in plants that have the most hydroxyl (OH) groups (Diniyah & Lee, 2020).

Table 3. Alcohol content of Kombucha tea from several sources

| **Alcohol Level** | **Source** |
|---|---|
| 0,297-0,621% | (Pratiwi et al., 2012) |
| 1,61-5,12% | (Simanjuntak et al., 2016) |
| 0,7-1,3% | (Kapp & Sumner, 2019) |
| 0,2-3,5% | (Jakubczyk et al., 2020) |
| 0,06-1,95% | (Tan et al., 2020) |

Rosella leaves contain several phytochemical contents. The contents of rosella leaves include 0.23 mg/g flavonoids, 0.125 mg/g phenolics, 0.13 mg/g saponins, 0.12 mg/g alkaloids, and 0.17 mg/g tannins. Apart from that, the nutritional content of herbal roselle leaves is 86.2% water content, 1.7–3.2% protein, 1.1% fat, 10% fiber, 0.18% calcium, 54 mg/100 g ascorbic acid (Mahadevan *et al.,* 2009; Nurnasari & Khuluq, 2017). The alkaloid content in roselle can be seen at the absorption wave of 1142.92 cm-1. The C-N group detected at an absorption wave of 1142.92 $cm^{-1}$ is possibly an alkaloid compound with a piperidine base structure as in research conducted by Fachriyah *et al.* (2018). Kareru *et al.,* (2008) stated that saponin has absorption of the infrared functional groups OH, CH, C=C, C=O, and C-O-C. It can be seen in Figure 2 that the absorption of the OH group of rosella kombucha tea is more stretched compared to green tea, this is also caused by the saponin content in rosella kombucha tea. Uptake of saponin oligosaccharides with saponins.

Kombucha drink products are fermented tea drink products using microbes as fermentation agents. Nile pH in kombucha ranges between 3. 0 - 5. 5 depending on the fermentation time. The pH value of kombucha is influenced by the accumulation of acids formed during fermentation (Saputra *et al.,* 2017). The results obtained in this research were that there was a pH difference between green tea and rosella kombucha. The pH value of the rosella kombucha is 2.63 while the green kombucha is 3.32. The pH value in rosella kombucha is lower because the ascorbic acid content in rosella is 54 mg/100 g (Mahadevan et al., 2009; Nurnasari & Khuluq, 2017).

# 4 CONCLUSIONS

In FTIR analysis, green tea and rosella kombucha showed absorption at wave numbers indicating the presence of certain functional groups. The difference in stretching of the O-H groups between the two is caused by the influence of microbiological activity during fermentation. Rosella also provides additional bioactive compounds, such as flavonoids and phenolics, which enrich the chemical profile of rosella kombucha. The pH value is lower in rosella kombucha because of the high ascorbic acid content in rosella. Both contain alcohol as a result of fermentation. This shows the potential of rosella kombucha as a functional drink rich in bioactive compounds with broader health benefits, but requires further studies for more in-depth exploration. The research results show that green tea kombucha has the functional groups C-N amine, C=C alkene, C=C (3-fold) alkyne, and O-H alcohol, while the functional groups from rosella kombucha tea produce 5 functional groups, including the C - O functional group ( Alcohols, ethers, carboxylic acids, esters ).

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